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PATENT

The information contained in the computer readable form of Application No. 09/215,035 was prepared through the use of the software program "PatentIn" and was identical to that of the paper copy. This amendment contains no new matter.

Attached hereto is a marked-up version of the changes made to the Specification by the current Amendment. The attached pages are captioned "**VERSION WITH MARKINGS TO SHOW CHANGES MADE.**"

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Paragraph beginning at line 2 of page 8 has been amended as follows:

Figure 1: **Nucleotide sequence** (SEQ ID NO:1) **and deduced amino acid sequence** (SEQ ID NO:2) **of the CAK1-9 cDNA.** The nucleotide sequence (upper line) and the deduced amino acid sequence (lower line) of the CAK1 cDNA is listed with nucleotide numbers at left. The translation of CAK1 starts at nucleotides 100-102 (ATG) and terminates at 1986-88 (TGA). The putative signal peptide is underlined and a typical hydrophobic sequence for GPI anchorage is double-underlined. A likely furin cleavage site RPRFRR is underlined and the cleavage site shown by an arrow. There are four potential N-linked glycosylation sites (in bold letters). A variant polyadenylation signal (AGTAAA) is present 22 base pairs upstream from the polyadenylation tail. The original p6-1 cDNA sequence spans nucleotides 721 to 2138.

Paragraph beginning at line 21 of page 8 has been amended as follows:

This invention relates to the discovery of an antigen, referred to herein as mesothelin, found on mesothelium, mesotheliomas, ovarian cancer cells and some squamous cell carcinomas. Previously, an antibody designated monoclonal antibody K1 was described which reacts with an antigen found on OVCAR-3 cells (from a human ovarian tumor cell line) having a molecular weight of 40 kD (kilodaltons). See, e.g. U.S. Patent No. 5,320,956. The antigen described and claimed here was unexpectedly obtained during an attempt to clone and sequence the K1 antigen. Mesothelin in its full-length form has an apparent molecular weight of about 69 kD and appears to be the precursor protein for the 40 kD K1 antigen. The K1 antigen itself proved difficult to

clone and our first attempts resulted in the cloning of two different intracellular proteins as mentioned above (see Chang & Pastan, Int. J. Cancer, supra). Though the existence of the K1 antigen was known, its cDNA was not routine to clone. First, we were not able to obtain sufficient amounts of it to clone. The methods used here were more laborious, but successful because unbeknownst to us the K1 antigen was derived from a larger molecule that we did not know existed. The DNA sequence and corresponding amino acid sequence for full-length mesothelin are set out in Figure 1 and in SEQ ID NOS:1 and 2 ~~Sequence I.D. Nos. 1 and 2~~, respectively.

Paragraph beginning at line 20 of page 55 has been amended as follows:

Thus, the above describes the molecular cloning of the CAK1 antigen which is found on mesothelium, mesotheliomas, ovarian cancers and some squamous cell carcinomas. We have designated this antigen mesothelin to reflect its presence on mesothelial cells. One unexpected feature of mesothelin is that its cDNA encodes a 69 kD protein, whereas the antigen present on OVCAR-3 cells, used to isolate MAb K1, has a molecular weight of ~40,000 Daltons. The DNA sequence and the deduced amino acid sequence of CAK1 is shown in Fig. 1. The cDNA is 2138 bp in length and contains an open reading frame of 1884 bp. The protein it encodes contains 628 amino acids with a calculated molecular weight of 69001 daltons. A homology analysis was performed with nucleotide or amino acid sequences and none was detected using EMBL-GenBank accessed by the GCG program. The protein contains four potential N-linked glycosylation sites N-X-S or N-X-T that are shown in bold letters. A typical signal sequence is not present at the amino terminus. However, a short hydrophobic segment is located 15 amino acids from the first methionine (Fig. 1). This sequence might function as a signal sequence for membrane insertion, because the protein is found on the cell surface and is inserted into microsomes during cell free translation. Also present is a putative proteolytic processing site, RPRFRR (SEQ ID NO:8), beginning at amino acid 293 (Fig. 1). This site is recognized by furin, a protease important in the processing of

several membrane proteins as well as in the activation of Pseudomonas and diphtheria toxins (Chiron, M.F., et al., J.B.C. 269(27):18169-18176 (1994)). The 40 kD form appears to be derived from a 69 kD precursor by several processing steps. These are summarized in Fig. 2. Initially, mesothelin is made as a 69 kD polypeptide with a hydrophobic tail which is probably removed and replaced by phosphatidylinositol (Chang, K., et al., Cancer Res. 52, 181-186 (1992)). After glycosylation at one or more of its four putative N-linked glycosylation sites, it is cleaved by a protease to yield the 40 kD fragment (or doublet) found on the surface of OVCAR-3 cells and a smaller (~31 kD) fragment. The latter could be released into the medium and/or further degraded. The amino terminal fragment has recently been detected in the medium of OVCAR-3 cells (our data). In transfected NIH 3T3 and MCF-7 cells, we find approximately equal amounts of 70 kD and 40 kD proteins. We originally detected the 40 kD form in OVCAR-3 and HeLa cells and did not notice a larger form. Reexamination of the OVCAR-3 and HeLa cell gels reveals a trace amount of the 70 kD precursor.